In the Specification

Applicants present replacement paragraphs below indicating the changes with insertions indicated by underlining and deletions indicated by strikethrough.

Please amend the title as follows:

NUCLEOTIDE SEQUENCES OF THE NUCLEOCAPSID (NP) GENE ~~AND PHOSPHOPROTEIN (P) GENES~~ OF A MALAYSIAN VELOGENIC NEWCASTLE DISEASE VIRUS STRAIN AF2240 ~~AND THE PRODUCTION OF THE NP AND P PROTEINS IN *ESCHERICHIA COLI*~~

Please replace the paragraph beginning on page 2, line 8, with the amended paragraph as follows:

The genes encoding for the HN (EMBL/Gen Bank/DDBJ accession No.~~X70092~~X79092), F (EMBL/Gen Bank/DDBJ accession No.AFO48763) and M (EMBL/Gen Bank/DDBJ accession No. AF060563) proteins of the NDV strain AF2240 have been completely sequenced by Tan *et al.* (1995), Salih *et al.* (2000) and Jemain, S.F.P. (1999) respectively. From the HN gene sequence of strain AF2240, it was quite clear that this strain is different from the other published NDV strains. The HN protein lacked the Arg (403) residue and contained 581 amino acids. At the time when the project was initiated, there was no information available on the coding sequences for the NP and P proteins of NDV strain AF2240. Therefore it remained a problem to prepare cDNA for the cloning of the NP and P genes of NDV.

Please replace the paragraph beginning on page 14, line 9, with the amended paragraph as follows:

The nucleotide sequences of the NP and P genes were determined by the ABI PRISM® automated sequencer, model 377. The recombinant plasmids, pTrcHis2-NP and pTrcHis2-P, were used as templates and the synthetic primers used in the sequencing reactions of the NP and P genes are as follows:

Please replace the paragraph beginning on page 15, line 18, with the amended paragraph as follows:

Total RNA was extracted using the ~~Trizol~~ TRIZOL® LS reagent (Gibco BRL, USA). Briefly, 250 µl of the virus infected allantoic fluid was mixed with 750 µl Trizol LS reagent and incubated for 5 min at room temperature. After incubation, 100 µl of 1-bromo-3-chloropropane (BCP) (MRC, UK) was added and the mixtures were mixed vigorously for about 15 s and again incubated at room temperature for 10 min. The mixtures were phase separated by ~~microcentrifugating~~ microcentrifuging at 13,000 xg for 15 min at 4°C (Jouan MR 1812, France). The RNA was then precipitated by adding 500 µl of isopropanol (Merck) to the aqueous phase and left at room temperature for 10 min. The precipitated RNA was microcentrifuged at 13,000 xg for 10 min and the pellet obtained was washed once with 75% (v/v) diethyl pyrocarbonate (DEPC) (Sigma, USA) treated ethanol (Hamburg). The pellet was dissolved in 20 µl of DEPC treated dH_2O.

Please replace the paragraph beginning on page 16, line 10, with the amended paragraph as follows:

For the amplification of the respective NP and P genes, another 20 µl of reaction mixture containing 1 U of ~~DyNAzyme~~ DYNAZYME™ EXT DNA polymerase (FINNZYMES), 1.5 mM of $MgCl_2$ and 1 x of reaction buffer was added to the top of the above cDNA mixture which was held at 94°C in the thermal cycler. The PCR profile for the amplification of NP gene comprising denaturation at 94°C for 30 s, annealing at 55°C for 50 s and extension at 72°C for 1 min for a total of 30 cycles. To ensure a complete synthesis of the PCR product, the extension step at 72°C was prolonged for 7 min after the last cycle. The PCR profile for the amplification of P gene was basically similar to that of NP gene, except the annealing step was carried out at 55°C for 30 s.

Please replace the paragraph beginning on page 16, line 20, with the amended paragraph as follows:

A total of 40 µl of the amplified PCR product was analysed on 1% TAE agarose gel. After the staining with ethidium bromide, the band with the correct size was excised from the gel

and purified with the ~~Wizard~~ WIZARD® PCR Preps DNA Purification System (Promega, USA) according to the manufacturer's procedures. After purification, 5 µl of the PCR product was again analysed with agarose gel electrophoresis to determine the recovery of the PCR product, which would be used in TA CLONING® ~~cloning~~.

Please replace the paragraphs beginning on page 17, line 2, with the amended paragraphs as follows:

TOPO® TA Cloning of NP and P genes

Four µl of the purified NP or P DNA fragments carrying an A overhang at their 3' ends was mixed with 1 µl of the pTrcHis2 TOPO® expression vector (~~Invitogen~~ Invitrogen, USA) and the ligation reaction was carried out at room temperature (25°C) for 5 min to form the desired recombinant plasmid.

Please replace the paragraphs beginning on page 18, line 20, with the amended paragraphs as follows:

Purification of NP fusion protein using ~~ProBond~~ PROBOND™ Column

Two hundred µl of LB medium containing 50 µg/ml ampicillin was cultured with 2 ml of overnight culture of transformant harbouring plasmid pTrcHis2-NP (carrying the NP insert without a stop codon), and the cells were grown to an OD_{600} of 0.6 to 0.8. Protein expression was then induced by adding 1 mM IPTG and the cells were grown for another 5 h. The cells were harvested by centrifugation at 2000 x*g* for 15 min at 4°C. The cell pellet was first resuspended in 10 ml of binding buffer (500 mM NaCl, 20 mM NaH_2PO_4, pH 7.8), then 100 µg/ml of lysozyme was added and incubated for 15 min on ice. The cells were lysed by sonication until the cell lysate is no longer viscous. The cell lysate was then treated with RNase and DNase I, both at a concentration of 5 µg/ml for 15 min at 30°C. The cell lysate was then centrifuged at 10,000 x*g* for 20 min to remove all the cell debris. The supernatant was collected and passed through a 0.45 µm filter. This cell lysate was incubated with the ~~ProBond~~ PROBOND™ resin (~~Invirogen~~ Invitrogen, USA) for 30 min and then allowed to drip through the resin. The column was washed with 10 ml of washing buffer (50 mM Imidazole, 500 mM NaCl, 20 mM NaH_2PO_4, pH 6.0), and the proteins were then eluted with 5 ml of elution buffer (500 mM Imidazole, 500 mM NaCl, 20

mM NaH_2PO_4, pH 6.0). The elute was collected as 1 ml fractions. Samples from each fractions were analysed on 12% SDS-PAGE to check the purity of the protein.